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American Chemistry Council Good Chemistry Makes It Possible

Via Messenger
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1200 Pennsylvania Avenue, NW
Washington DC 20460-001

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Attention: TSCA Section 8(e) Coordinator

RE: Ethylene Glycol- Follow Up to Preliminary Results in Bladder from One-Year Exposure in Male Wistar Han Rats

Dear Sir or Madam:

The American Chemistry Council Ethylene Oxide/Ethylene Glycols Panel submits this letter on behalf of certain of its members[1] as a follow up to the Panel's submission of January 12, 2005 to the TSCA 8(e) Coordinator. The Executive Summary of the completed study is attached.

If you have any questions, please contact me at 703-741-5613 or william_gulledge@americanchemistry.com

Sincerely,

8EHQ-05-15905

William Gulledge
Manager,
Ethylene Oxide/Ethylene Glycols Panel

Attachment: Summary of "Ethylene Glycol – 12-Month Dietary Toxicity Study in Wistar Han Rats."

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1 Companies sponsoring this study are: The Dow Chemical Company, Eastman Chemical Company, Equistar Chemicals LP, Huntsman Corporation, and Shell Chemical Company LP.

SUMMARY

The objective of this study was to assess the renal toxicity potential of ethylene glycol (EG) in male Wistar Han rats after 12 months dietary administration. The concentrations of EG and its metabolites, glycolic acid (GA) and oxalic acid (OA), in the blood, kidneys and urine, and the clearance kinetics of OA were also assessed. Additionally, the strain and age-dependence of clearance was evaluated in satellite groups of naïve male F-344 and Wistar rats. Benchmark dose (BMD) analyses were conducted for human health risk assessment purposes using compound-induced nephropathy and birefringent crystal data.

EG was given in NTP 2000 (lower protein) diet at 0, 50, 150, 300, and 400-mg/kg body weight/day (mkd) to groups of twenty male Wistar rats for 12 months. Ten rats per group (main group) were used to evaluate renal toxicity, five rats per group were used to evaluate metabolites, and five animals per group were used to determine renal clearance. Parameters assessed in the main group included cage-side and clinical observations, body weights, feed and water consumption, urinalysis, organ weights, gross necropsy, and histopathologic examination of kidneys and bladders. Strain and age dependence of OA clearance were assessed using four naïve Fischer-344 rats approximately 1-yr old and groups of five 9-12-wk old naïve Wistar and F344 rats. Ten sentinel animals were maintained in the study room for the 12-month duration of the study.

In-life treatment, necropsy, and clearance analyses were conducted at The Dow Chemical Company, Midland, Michigan. Histological staining of the urinary bladder and kidney slides was done by WIL Research. Microscopic histopathology evaluation was conducted by Gordon C. Hard, BVSc., Ph.D., D.Sc. Metabolic analyses were conducted by Richard Corley, Ph.D., Battelle Northwest. BMD analyses were conducted by the Sapphire Group.

One control rat died (day 307), no rats given 50-mkd died, one rat given 150-mkd died of a spontaneous rat lymphoma (day 267), and four rats given 300-mkd died (on day 111, 207, 213, or 221) with a fifth rat at that dose level declared moribund on day 138. At 400-mkd, 4 rats died spontaneously or were humanely euthanized in a moribund state (on day 43, 154, 187, or 193). On day 203, the sixteen remaining animals given 400-mkd were humanely euthanized because of excessive body weight loss. The mortality at 300 and 400-mkd was considered treatment-related.

All rats given 300-mkd that died or were declared moribund prior to study termination had gross findings on the bladder and four of them had gross findings on the kidney,

with the cause of death attributed to sequelae of urinary obstruction. The underlying cause of death/moribundity determined following gross and histopathologic examination was related to effects on the urinary bladder or kidney as described below.

During the study, animals given 300 or 400-mkd had occasional treatment-related absent/decreased feces, blood in the cage, red urine, red perioral and perinasal soiling, and/or perineal soiling. There were no treatment-related clinical signs at 50 or 150-mkd.

Rats given 300 or 400-mkd had treatment-related decrements in body weight and body weight gain. The differences from controls occurred within the first few months in animals given 400-mkd and were first statistically identified on day 141, when body weights and body weight gains were 12.7% and 21.4% less than controls, respectively. On study day 197 at 400-mkd, body weights were 20.1% less than controls and body weight gains were 31.3% less; therefore, the remaining rats at this dose were humanely euthanized on study day 203 because of excessive body weight loss. Body weights for rats given 300-mkd were typically lower than controls by mid-study, with all but one animal usually having body weight less than the control mean. These effects were considered related to treatment but were not statistically identified because of the large standard deviations. The body weight effects for rats given 300-mkd occurred gradually, and on study day 141, body weights and body weight gains were 5.2% and 8.4% less than controls, respectively. After day 141, differences from controls in body weights and body weight gains leveled off. No body weight effects occurred at 50 or 150 mkd.

Feed aversion/scratching occurred at ≥ 150-mkd, which was reflected in the smaller sample size as these feed consumption data were not collected. Rats given 400-mkd had treatment-related decreases in feed consumption at every time point through termination on day 203, which were typically statistically identified from study day 106. There were no treatment-related effects on feed consumption for rats given 50, 150, or 300-mkd.

Water consumption was analyzed near the 12-month end of the study. Rats given 300-mkd had a treatment-related increase in water consumption of 151% of controls. There were no treatment-related effects on water consumption for animals given 50 or 150-mkd.

After 12 months, decreased urinary pH occurred in all treatment groups but was not considered adverse but rather likely due to the presence of metabolic products of EG.

Animals given 300-mkd had increased urine volume and concomitantly decreased urine specific gravity compared to controls, which correlated with the increase in water consumption. The more dilute urine in the 300-mkd group might also explain the finding that less animals in this group had decreased urinary pH than in the 150-mkd group. Analysis of urinary crystals demonstrated treatment-related effects at all EG doses, with the proportion of crystals that were composed of calcium oxalate increasing with increasing EG dose, and those composed of phosphate decreasing with increasing EG dose. This compositional effect was considered a metabolic consequence of EG exposure as no adverse effects were seen from the crystals observed in the 50 or 150-mkd groups.

Increases in absolute and relative kidney weights occurred in animals given 300 or 400-mkd. These were not statistically identified at 300-mkd and were not statistically analyzed at 400-mkd, but were considered treatment-related. There were no contemporaneous controls for the animals given 400-mkd since they were sacrificed early, but remarkable increases occurred in their absolute and relative kidney weights versus all other groups that went to term, although rats at 400-mkd weighed much less.

Treatment-related gross pathological observations occurred in animals given 300 or 400-mkd and were primarily confined to the kidney and urinary bladder, with secondary treatment-related observations occurring in the lung. For rats given 300-mkd, of 15 rats examined, 7 had findings on the kidney and 8 had findings on the urinary bladder. For rats given 400 mkd, of 20 rats examined, 17 had findings on the kidney and 10 had findings on the urinary bladder. The most relevant observation in the 300-mkd group was the presence of calculi in the bladder (and sometimes the renal pelvis or ureter) in 8 of the total 15 rats examined. This also occurred in 8 of 20 rats at 400-mkd. Calculus formation in the urinary bladder was usually accompanied by dilatation of the bladder and, for the 5 unscheduled deaths at 300-mkd, hemorrhage of the bladder wall, usually with ascites or other edematous change. Three animals given 300-mkd had calculi in the renal pelvis. Almost all rats at 400-mkd showed signs of kidney and/or urinary bladder involvement, including a roughened kidney surface, renal pelvic dilatation, thickened bladder wall, and calculi in the renal pelvis, ureter, or bladder. Of the four unscheduled deaths occurring before early termination of this group, three were observed to have hemorrhage of the bladder wall. Some animals given 400-mkd also had decreased body fat, increased size of the renal lymph nodes, and calculus in the ureter or a dilated ureter. Treatment-related gross pathological effects on the lung, which were less frequent and considered secondary sequelae to effects on the kidney, consisted of a mottled appearance in four rats given 400-mkd. Gross pathological findings of congestion and edema that occurred in the lungs of several animals given either 300 or 400-mkd may have been associated with agonal effects as these animals were found dead. The decrease in body fat observed for five animals given 400-mkd was considered reflective of the general decrease in body weight demonstrated by animals at this dose level. Ureter dilatation and calculi observed in two animals given 400-mkd are considered secondary to effects on the kidney and bladder. The increased size of the renal lymph nodes was considered a secondary consequence of the renal findings observed in eight animals given 400-mkd.

Histopathological examination showed that a compound-induced nephropathy associated with crystalluria affected the majority of the animals at 300-mkd, and all of those given 400-mkd. None of the renal alterations associated with EG exposure (basophilic foci of crystalluria-related nephropathy, tubule dilatation, birefringent crystals particularly in the pelvic fornix, renal pelvic dilatation, or transitional cell hyperplasia) were observed in the rats given 50 or 150-mkd, establishing the latter dose-level as a NOAEL.

Calculi, up to 2-mm diameter, were found in the bladder, and sometimes in the renal pelvis, at the two highest doses. Since the cause of early death for 3 animals at 300-mkd was unlikely to be related to the extent of the compound-associated kidney changes, which were less than end-stage, bladder tissue from some animals in each group was examined. Histological findings in the bladder and ureter correlated well with the observations of calculi. The basic change was simple transitional cell hyperplasia, progressing to acute inflammation and hemorrhage in severe cases. In animals dying before scheduled termination in groups given 300 or 400-mkd, the acute inflammation and hemorrhage of the bladder wall was a consistent finding in all but one case, and considered to be related to the cause of death. Such severe bladder pathology was often accompanied by a necropsy record of ascites or other edematous change, suggesting that infection via the bladder wall and septicemia may have been the terminal event.

The renal clearance rates of ^{3}H-inulin and ^{14}C-oxalate were evaluated in the control, 50, 150, and 300-mkd groups as well as naïve, male, young Wistar and F344 rats (9-12 weeks of age) and naïve, male, old F344 rats (47-56 weeks of age) to obtain information on renal clearance capability in rats of different strains and ages.

There were no treatment-related changes in oxalate or inulin clearance in the male Wistar rats after 12-months. Clearance ratios were 0.82 for controls and 0.73-0.87 for the 50, 150, and 300-mkd groups. Oxalate clearance rates ranged from 3.91-4.79 ml/min/kg bw.

Clearance ratios were not significantly different for the young versus old Wistar rats and varied from 0.59 to 0.82, respectively. While these results suggest an age-dependent increase in oxalate clearance, the actual clearance of oxalate was found to be quite constant with age (3.80-3.91). This variation in oxalate/inulin clearance ratios was most probably due to an age-dependent decrease in inulin clearance. In contrast, the ratio of oxalate to inulin clearance was lower in the young versus old F344 rats (0.70 vs. 0.81; not statistically significant), while the oxalate clearance rate was higher for the young versus old F344 rats (6.06 versus 4.56 ml/min/kg, respectively; not statistically significant), suggesting a higher rate of oxalate and inulin clearance in the young versus old F344 rats.

The only statistically identified difference in the rate of oxalate clearance was between the naïve young Wistar and F344 rats, which was significantly higher in the F344 rat. The clearance of oxalate was slightly higher in the old F344 versus Wistar rat (4.56 vs. 3.91 ml/min/kg, respectively; not statistically significant). Although old male F344 rats also have a reduced capacity for clearance of OX, similar to that of young and old male Wistar rats, the strain differences in sensitivity are maintained even through one year of exposure.

Blood, urine, and kidney samples collected from the metabolite satellite group of Wistar rats exposed for 12 months at 0, 50, 150, or 300-mkd EG were analyzed for EG, glycolic acid (GA), and oxalic acid (OX). A section of kidney from each animal in the 400-mkd group that was sacrificed on study day 203, and a section of kidney from all main study animals at 12 months, were also analyzed for EG, GA, and OX. There was a contaminant in the derivatization agent used for the analysis of EG in all samples except urine, which was analyzed directly. Thus for EG, only the urine data are reported.

The clearance of EG in urine followed a linear dose-response relationship across all dose levels. A linear increase in urinary clearance of GA was observed at 50 and 150 mg/kg-day while a disproportionate (non-linear) increase was observed at 300-mkd. Urinary clearance of OX was similar to controls across all dose levels. In the kidneys, there were no differences in the concentrations of GA and OX at dose levels up to

150-mkd, compared with controls. However, there were clear non-linear increases in the concentrations of GA and OX at dose levels of 300 and 400-mkd. Concentrations at 400-mkd reached an average of 14 µg/g and 18,800 µg/g for GA and OX, respectively, with some animals having considerably higher concentrations of each metabolite than average. In fact, OX concentrations, when expressed as calcium oxalate, accounted for an average of 2.9% of the total kidney weight (with one animal approaching 11.2%) in the animals exposed to 400 mg/kg-day and sacrificed early in the study. As with the results from the kidneys, the concentrations of GA in blood were not significantly different from controls up to 150-mkd. At 300-mkd, the concentrations in blood were approximately 3.3-fold higher than controls although the concentrations were all <10 µg/g regardless of dose level. The concentrations of OA in blood were also similar across all dose levels, averaging 3.7-5.1 µg/g. These results were expected from the low solubility of OA at physiological pH in aqueous media.

BMD analyses were conducted using compound-induced nephropathy and birefringent crystal data from Wistar rats chronically exposed to EG for the purposes of defining a dose corresponding to an extra risk of 5% (BMD05) and its lower confidence limit (BMDL05). The respective BMD05 and BMDL05 values using incidence and severity were
170 mg/kg-day and 150 mg/kg-day for compound-induced nephropathy, and 170 mg/kg-day and 160 mg/kg-day for compound-induced birefringent crystals.

In conclusion, chronic dietary administration of EG to male Wistar Han rats for 12 months resulted in:

- The maximum tolerated dose (MTD) was exceeded at 400 mkd as excessive body weight loss at this level necessitated early termination and there were histopathologic manifestations of marked renal toxicity.
- The no-observed-adverse-effect level (NOAEL) was 150 mkd based on the absence of manifestations of systemic or renal toxicity at this dose.
- A no-observed-effect level (NOEL) was not established as decreased urinary pH and increased urinary oxalate crystals occurred at all treatment levels (≥ 50 mkd), however, these were not considered adverse but rather normal metabolic/physiological consequences of chronic EG exposure.
- There were no treatment-related effects on oxalate or inulin clearance.

- Urinary clearance of OX was similar to controls across all doses, that of EG followed a linear dose-response relationship, and that of GA was linear between 50 and 150-mkd, with a disproportionate non-linear increase at 300-mkd.
- Kidney concentrations of GA and OX were similar to controls at doses up to 150-mkd. However, there were clear non-linear increases in the kidney concentrations of GA and OX at dose levels of 300 and 400-mkd.
- The respective BMD05 and BMDL05 values using incidence and severity data were170 mg/kg-day and 150 mg/kg-day for compound-induced nephropathy, and 170 mg/kg-day and 160 mg/kg-day for compound-induced birefringent crystals.